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Introduction: Cytochrome P 450 enzymes (CYPs) were presumed to play a role in the oxidation of intermediate metabolites of busulfan (Bu). In vitro elucidation of involvement of CYPs in the oxidation of Bu metabolites is cumbersome due to the volatile nature of tetrahydrothiophene and nonavailability of sensitive quantitation methods. This study is aimed at exploring the association of *CYP2C9, CYP2C19, CYP2B6, FMO* genotypes, and sulfolane (Su) levels in children undergoing hematopoietic stem cell transplantation (HSCT).The relation of genotypes with the outcomes of HSCT was also explored.

Patients (or Materials) and Methods: Sixty-six children receiving IV Bu-based myeloablative conditioning regimen were genotyped for common functional variant alleles in *CYP2C9 (*2* and **3), CYP2C19 (*2* and **17), FMO3 (rs2266780, rs2266782* and *rs1736557)* and *CYP2B6 (*5* and **9)*. Plasma levels of Bu and its metabolite Su were measured after dose 9 from a subset of 44 patients for whom plasma samples after dose 9 were available. The ratio of Bu to Su was taken as a metabolic ratio (MR) to compare among genotype groups. The MRs (Bu/Su), Bu and Su levels between different genotype groups were compared using nonparametric tests. The distribution of age, and gender between the groups was compared using *t* test and chi-square test, respectively. Cumulative incidence of overall survival and event-free survival were estimated using Kaplan-Meier curves and log-rank test was used to compare the difference between genotype groups or groups divided on the basis of MR, in a univariate analysis. Multivariate analysis was performed using cox-regression analysis.

Results: Higher metabolic ratios (MRs, Bu/Su) were observed in *CYP2C9*2 and *3* allele carriers (mean [SD], 7.8 [3.6] Vs 4.4 [2.2]; $P = 0.003$). Lower event-free survival was seen in patients with MR above the median 5 (40% vs 79%; $P = 0.009$) and carrying reduced function alleles of *CYP2B6* (40% vs 84%; $P = 0.005$).

Conclusion: This study suggests the role of the CYP2C9 in the oxidation reactions of THT and *CYP* genotypes along with Bu MRs to be important at predicting outcomes of Bu based myeloablative conditioning before HSCT.

Disclosure of Interest: None declared.

PP122—REGULATION OF HUMAN LEUKOCYTE ANTIGEN EXPRESSION AND NEVIRAPINE-INDUCED ADVERSE DRUG REACTIONS IN A MALAWIAN HIV-POSITIVE POPULATION

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Introduction: Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) widely used in antiretroviral therapy (ART) in sub-Saharan Africa. Approximately 5% of patients receiving NVP-containing regimens develop hypersensitivity reactions (HSRs). These can manifest as more severe reactions including Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), and hepatotoxicity. Various human leukocyte antigen (HLA) alleles have been associated to NVP-induced HSRs in populations of differing ethnicity. Carriage of *HLA-C*04:01* is associated with an increased risk of SJS in our Malawian cohort. We investigated whether expression levels of *miR-148a*, a microRNA known to regulate *HLA-C* expression, differ in serum samples from hypersensitive and tolerant patients. The aim of the study was to identify the role of posttranscriptional regulation of *HLA-C* expression in HLA-associated NVP-induced HSRs.

Patients (or Materials) and Methods: A total of 1117 HIV-positive patients in Malawi treated with a NVP-containing regimen were recruited prospectively. Of these, 57 patients developed NVP-induced HSRs. MicroRNA expression was analyzed using TaqMan probe–based qPCR in available serum samples of 41 tolerant and 33 hypersensitive patients. Expression levels were compared in hypersensitive patients at baseline (n = 19) and during the acute phase of the reaction (n = 26). Tolerant baseline (n = 25) and week 6 (n = 27) samples were used as controls. Data were analyzed using nonparametric tests.

Results: There was no significant difference in the baseline expression of *miR-148a* between tolerant and hypersensitive patients. In our cohort, *miR-148a* expression showed a 4.6-fold increase in acute hypersensitive samples, when compared with baseline hypersensitive ($P = 0.008$). This was not observed in samples from tolerant patients.

Conclusion: Our study has identified an increase in *miR-148a* expression levels in serum samples from Malawian nevirapine hypersensitive patients during the acute phase. The reason(s) for the change in expression during the acute phase are unclear but may be related to the ongoing inflammation associated with a HSR. Further investigation is required to elucidate the mechanism of the rise in microRNA levels and any implications this may have on the severity and duration of the HSR.

Disclosure of Interest: None declared.

PP123—TARGET GENE EVALUATION OF TWO MIRNAS DIFFERENTLY EXPRESSED IN FOCAL AND NON-FOCAL BRAIN TISSUE OF THERAPY-RESISTANT EPILEPSY PATIENTS

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Introduction: Resistance to anticonvulsants affects one third of all epilepsy patients. Limited bioavailability of the drug at the target site caused by increased expression of efflux transporters on the blood brain barrier or alterations of target genes as well as seizure-induced neural reorganization are potential mechanisms for therapy resistance. There is increasing evidence that expression of microRNAs (miRNAs) is deregulated in neuronal disorders. We hypothesize that an altered miRNA regulation of target genes is involved in drug resistance in epilepsy.

Patients (or Materials) and Methods: Hippocampal focal and cortical nonfocal brain tissue samples from 13 patients diagnosed with MTS (mesial temporal sclerosis) who underwent neurosurgery have been screened for miRNA expression using TaqMan® low-density arrays. To compare miRNA expression between brain regions, a Mann-Whitney U test was performed using R (Bioconductor). In silico approaches for both a hypothesis-based (efflux-transporter and target gene) as well as a hypothesis-free approach were used

to filter for potential phenotype-relevant target genes. After cloning 3'-UTR sequences containing predicted miRNA binding sites into psiCHECK2 or pmiRGLO vectors reporter gene assays were performed to confirm RNA interference of selected candidate miRNAs with their respective target genes.
Results: Of 754 miRNAs, 201 were detected in both tissue types. Two miRNAs were differentially expressed in the hippocampus relative to the cortex (miR-34c-5p: 7.2-fold higher [q = 0.01], miR-212-3p: 3.8-fold lower [q = 0.01]). Bioinformatic analysis and filtering for target genes identified 9 genes important for drug efflux, neuronal regulation, and signal transmission. Reporter gene experiments confirmed 3 target genes posttranscriptionally regulated by miR-34c-5p (GABBR2, GABRA3, GRM7) and 3 target genes regulated by miR-212 (ABCG2, SOX11, ADCY1).
Conclusion: Differential regulation of 2 miRNAs could contribute to an altered function of several genes resulting not only in an imbalanced neuronal excitability but also in impaired neural differentiation and accelerated drug export. These data suggest multifactorial alterations involving miRNA-mediated regulation leading to pharmacoresistance in epilepsy.
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PP124—PHARMACOKINETICS OF TOLPERISONE IN RELATION TO CYP2C19 GENOTYPES

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Introduction: Tolperisone, which is indicated in the treatment of acute muscle spasms in back pain and spasticity in neurologic diseases, is a centrally acting muscle relaxant. Although the metabolism of tolperisone is primarily mediated by CYP2D6, CYP2C19, CYP1A2, and CYP2B6 are also involved in the biotransformation of tolperisone. Among 3 drug-metabolizing enzymes, CYP2C19 is a highly polymorphic enzyme. The aim of this study was to investigate the effects of CYP2C19 genetic polymorphism on the pharmacokinetics of tolperisone.
Patients (or Materials) and Methods: Twenty-six healthy Korean subjects were selected and divided into 3 different groups according to CYP2C19 genotype, CYP2C19EM (CYP2C19*1/*1, n = 12), CYP2C19IM (CYP2C19*1/*2 or *1/*3, n = 7), and CYP2C19PM (CYP2C19*2/*2, *2/*3 or *3/*3, n = 7). After overnight fasting, each subject received a single 150-mg oral dose of tolperisone. Blood samples were collected up to 12 hours after drug intake, and plasma concentrations of tolperisone were measured by using LC-MS/MS analytical system.
Results: Cmax in CYP2C19PM group was significantly higher than that in CYP2C19IM and CYP2C19EM ($P = 0.0017$ for all). AUCinf in CYP2C19PM was also significantly higher than that in CYP2C19IM and CYP2C19PM group ($P < 0.001$ for all). Corresponding values for tolperisone in CYP2C19EM and IM groups were almost similar ($P > 0.05$). Apparent oral clearance (CL/F) of tolperisone in CYP2C19PM group was 84% lower than that in CYP2C19IM group (618 [379] vs 2900 [1343] L/h; $P = 0.0010$). Differences in other parameters of tolperisone between 3 genotype groups were not statistically significant.
Conclusion: In Korean healthy subjects, pharmacokinetics of tolperisone are not only influenced by CYP2D6 genotypes but also influenced by CYP2C19 genotypes. Particularly, CYP2C19PM subjects had markedly increased plasma concentration of tolperisone compared with CYP2C19EM or CYP2C19IM subjects.
Disclosure of Interest: None declared.

PP126—IN-VITRO REACTIVITY OF DRUG-SPECIFIC T-CELLS FROM A HLA-A*31:01 POSITIVE CARBAMAZEPINE HYPERSENSITIVE PATIENT

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Introduction: Carbamazepine (CBZ) causes hypersensitivity reactions in a small proportion of patients. There is strong evidence that specific human leukocyte antigen (HLA) alleles are associated with a higher risk of developing CBZ-induced hypersensitivity. HLA-A*3101 represents the latest example and is associated with several clinical phenotypes of CBZ-induced hypersensitivity in Caucasian and Japanese patients. In this study, we aimed to determine whether HLA-A*31:01 is functionally implicated in the development of a drug-specific immune response in our patient.
Patients (or Materials) and Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from a patient with CBZ hypersensitivity and the presence of drug-responsive T cells confirmed in vitro using the lymphocyte transformation test (LTT). The study was approved by the local ethics committee and informed consent was obtained from the patient. Drug-specific T cells were enriched in a 4-week induction culture and their reactivity tested with enzyme-linked immunospot (ELISpot) technique and 51Cr-release assay. T-cell clones (TCCs) were generated by serial dilution; characterization included CD phenotype, HLA restriction and cytokine profile.
Results: PBMCs responded to CBZ in the LTT with a stimulation index (SI) of 15.9. After the 4-week enrichment culture, T-cells were shown to secret Interferon-γ (IFN-γ) and kill 51Cr-loaded target cells when exposed to CBZ. Thirty-two CBZ-specific TCCs were generated; they secreted IFN-γ, interleukin-13 and cytolytic molecules such as granzyme B, perforin, and FasLigand. The majority of TCCs were CD4+ and T-cell activation was restricted by HLA class II alleles, i.e. HLA-DR and -DP. These TCCs proliferated in the presence of both CBZ and antigen presenting cells (APCs) expressing HLA-A*31:01 and HLA-DRB1*04:04, but also in the presence of HLA-DRB1*04:04+ APCs lacking HLA-A*31:01. HLA-DRB1*04:04 is known to be part of a common haplotype with HLA-A*31:01 in Caucasians.
Conclusion: We were able to stimulate a secondary immune response to CBZ in vitro using lymphocytes from a HLA-A*31:01+ hypersensitive patient. CBZ-specific T cells of CD4+ phenotype were restricted by HLA class II alleles, and proliferated in the presence of CBZ and HLA-DRB1*04:04+ A*31:01- APCs revealing that a common haplotype may contribute to the multi-clonal response seen in patients with CBZ hypersensitivity. Further studies are needed to confirm the association.
Disclosure of Interest: None declared.

PP127—CYP4F2 AND APOE CONTRIBUTION IN ACENOCOUMAROL DOSING BASED ON GENOTYPE: A COMPARISON OF TWO ALGORITHMS

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Introduction: Two algorithms for acenocoumarol stable dose prediction have been recently published. The first, developed by the EU-PACT group includes demographic (age, sex, weight, height, amiodarone cotreatment) and genetic variables (genetic variants in *CYP2C9* and *VKORC1* genes). The second one has been developed by our group (HULP algorithm) in a cohort of 147 patients with thromboembolic disease (VTD), including clinic-demographic